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REPORT OF BUREAU OF ANIMAL INDUSTRY

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During the past fiscal year (July 1, 1952 - July 1, 1953) a total of 237 samples of commercially produced pullorum whole-blood antigen has been examined for formaldehyde content, concentration of cells, sensitivity, and dye content. Two hundred and twenty-two of the samples were found to be satisfactory while 15 were rejected. Only a few samples of T.G. type antigen were received during the past year. Approximately one-half of the whole-blood antigens tested have been of the polyvalent type.

Five commercially-produced pullorum tube antigen samples were tested for cell concentration, sensitivity, and phenol content and all passed. One state-produced tube antigen was submitted from Ohio for examination and this antigen was found to be satisfactory.

A total of 4,800 cc. of K type pullorum antigen and 360 cc. of concentrated pullorum tube antigen was supplied to the North Central States as shown in Table 1. Most of the whole-blood antigen was supplied to State Officials for check testing purposes. Several of the States requested the antigens for comparative studies of whole-blood and tube methods of testing turkeys for pullorum disease.

Table 1. B.A.I. Pullorum Antigen Supplied to North Central States during Fiscal Year July 1, 1952-July 1, 1953

State	K Antigens (cc.) S	V	Poly.	Tube Antigen (cc.) S	V	Poly.
Illinois	1,960	-	40	-	-	-
Indiana	20	-	20	-	-	-
Iowa	-	-	-	-	-	-
Kansas	260	240	240	60	40	40
Michigan	40	-	40	20	-	20
Minnesota	40	40	40	20	-	20
Missouri	40	-	40	-	-	-
Nebraska	360	220	480	-	-	-
North Dakota	-	-	-	40	-	-
Ohio	40	-	40	20	-	20
South Dakota	480	-	-	-	-	-
Wisconsin	40	40	40	20	20	20
TOTAL	3,280	540	980	180	60	120

Studies of the relative keeping quality of K and T.G. type antigens have been completed during the past year. The initial six month expiration date placed on these antigens was based on the observations made shortly after the development of the antigens that some lots, for causes unknown, tend to deteriorate on aging. It has been recognized from the beginning of the work with pullorum antigens that a longer dating would offer certain advantages to both the producers and users. For this reason the keeping qualities of commercially produced stained antigens have been investigated on several occasions.

Commercial antigens were first examined at the time they were submitted for approval which of course was shortly after they were produced. The antigens that passed were then held under refrigeration for a period of one year and retested. Antigens produced routinely in this laboratory and subsequently stored were also examined. The retests included determinations of density, formaldehyde content, color, and sensitivity.

Results indicated that the keeping quality of K antigen is superior to that of T.G. and that the expiration date on K antigen could be safely extended to nine months. Effective March 1, 1953, for a trial period of one year, firms are being permitted to use an expiration date not to exceed nine months from the date of production for K and K polyvalent pullorum stained antigens. The production of T.G. pullorum stained antigen was discontinued effective July 1, 1953.

The T.G. formula for the production of pullorum whole-blood antigen was developed in 1940 because of non-pullorum type reactions which were frequently noted in using the old regular type antigen prepared on a simplier medium. The T.G. medium also provided greater yields of the organism. The old regular type medium provided about 16 cc. of finished antigen from each 100 cc. of medium, while through the use of T.G. type medium the yield was increased to approximately 60 cc. of finished antigen from 100 cc. of medium. The K type medium developed in 1947 by the Bureau was found to yield an antigen of improved specificity, increased sensitivity, and better keeping qualities. Yields on K type medium are comparable to those on T.G. Through other media improvements it may be possible to add further to the keeping quality of the finished antigen.

Antigen strains designated as B.A.I. 4 and Canadian 296 do not grow well on K type medium. Efforts are presently being made to increase the yields of these strains through serial passage of the stock cultures on K type medium. Strain 4 has recently been found to contain some antigenic factor XII_2 (variant). However, in addition this strain contains well-developed XII_3 factor and is satisfactory for the production of standard K type antigen.

Recently studies were in progress on the effect of the salt ammonium sulfate ($(NH_4)_2\ SO_4$) on several antigenic types of S. pullorum. The observation was made that suspensions of standard antigenic strains were rapidly flocculated

and sedimented by lower concentrations of the salt than were variant and intermediate type strains. Thus, the supernatant fluid of standard type suspensions was cleared of cells following centrifugation. The supernates of variant suspensions remained very turbid with the sedimentation of some cells while the supernates of intermediate type strains were only partially cleared following centrifugation.

The sedimentation effect of ammonium sulfate on the various antigenic forms of S. pullorum could be rather closely correlated with serological typing results, indicating that there exists a sedimentation difference of the various antigenic forms of S. pullorum in ammonium sulfate solutions, possibly due to chemical or physical differences in XII_2 and XII_3 type cells.

Briefly, the technic used is as follows: Cultures to be typed are streaked on deep slants of K type medium. After a 24 hour incubation period at 37°C. the growth is harvested in 2-3 cc. of formolized phosphate buffer (pH 6.4) and for exacting results standardized using optical methods to a density of 100 X tube No. 1 of the McFarland scale. To 0.5 cc. of this suspension in a serological tube, 8 cc. of aqueous ammonium sulfate solution (330 Gm./L.) are added. The tubes are incubated in a 55°C. water bath for five minutes and subsequently centrifuged for 15 minutes at 2,400 r.p.m. Supernatant turbidity readings are then made. A clear supernate or only a trace of turbidity indicates a standard type culture; a very dense supernate with some sedimentation indicates a variant type culture; intermediate strains show varying degrees of turbidity dependent on the balance of XII_2 and XII_3 type cells.